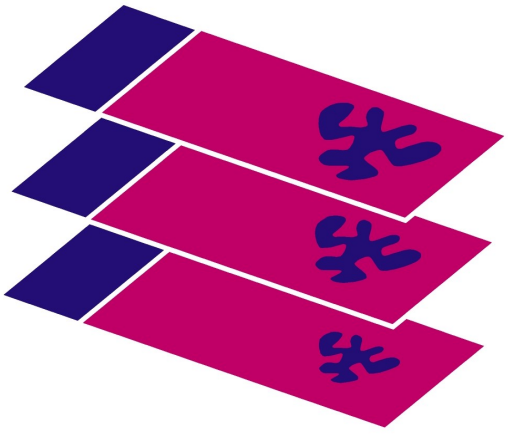

3DHISTECH

3D Module 1.11

User's Guide

June 25, 2008

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Font Types and Symbols

Example	Abbreviation or term that is explained in the section Terms and Abbreviations (page 5).
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task.
Important!	Contains an important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	Contains a suggestion about using the application in some other way or to some interesting purpose.

Terms and Abbreviations

3D Project	A set of digitized slides, created from consecutive cuts from the specimen.
3D Object	A virtual reconstruction from the specimen. The 3D object is not saved; MIRAX 3D calculates the rendered object when you open the project.
Voxel	Volumetric pixel. Analogous to a pixel (representing 2D image data), a voxel represents 3D image data: it represents a value in a grid in three-dimensional space.

Keyboard and Mouse Shortcuts in MIRAX Viewer and its Modules

Keyboard shortcut	Description
Up	Moves the slide up
Down	Moves the slide down
Left	Moves the slide to the left
Right	Moves the slide to the right
+	Increases the magnification
-	Decreases the magnification
F1	Displays the shortcuts.
F2	Displays the entire slide in the active window.
F3	1x magnification
F4	2x magnification
F5	5x magnification
F6	10x magnification
F7	20x magnification
F8	40x magnification
F9	Changes the magnification of the preview screen
F11	Turns full screen view on/off
Ctrl+T	Turns slide tree on/off
ESC	Returns from full screen mode to normal mode
Ctrl+M	Shows or hides the magnifier window in the top-right corner of the screen.
Ctrl+P	Shows or hides the slide preview panel.
Ctrl+V	Switches between the Fit and Full View modes in the upper preview window on the slide panel: • In Fit mode, the preview window shows only the digitized specimen cut. • In Full View mode, the preview window shows the entire slide.

1 About the 3D Module

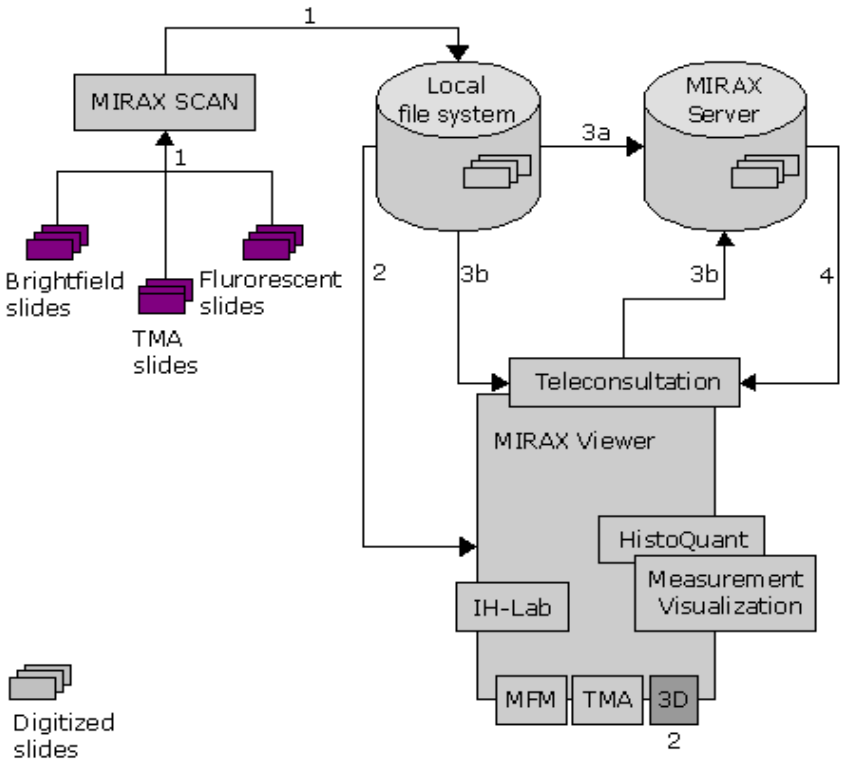
3D is an optional module for MIRAX Viewer. It allows you to reconstruct tissue sections as virtual three-dimensional objects.
MIRAX 3D requires a MIRAX digital slide set for the virtual reconstruction, no other special tools or techniques are needed. Compared to the 3D images created by confocal microscopes, which are simply summations of the scanned optical slices, MIRAX 3D creates virtual 3D objects, which you can be moved, rotated and virtually re-cut. This method allows for the investigation of the inner structure of the recomposed object rather than focusing on its surface only.

Important!

The greatest care should be taken when cutting serial tissue sections in order to avoid any deformation of the slices, since damaged or distorted sections might interfere with the virtual 3D reconstruction of the original specimen.

1.1 3D in the MIRAX Product Family

The following figure shows how the 3D module fits into the MIRAX product family as an integral part of MIRAX Viewer.



- Slides are digitalized with the MIRAX SCANNER. Each digitalized slide consists of an MRXS file plus a folder with the same name as the MRXS file.

  If you have a series of cuts from a given specimen, each slide is digitalized separately.

- Digitalized slides can be inspected with MIRAX Viewer. You can reconstruct the specimen as a virtual object using the 3D module.

  Annotations from other modules, such as HistoQuant, are ignored during 3D reconstruction.

- If you have the Teleconsultation module, you can upload the slides to a MIRAX Server.

- Slides on a MIRAX Server can be shared in a teleconsultation.

For further information about other modules of MIRAX Viewer, see ***MIRAX Viewer - User's Guide***.

1.2 About MIRAX Slides to be Used in 3D Projects

When working with the 3D module of MIRAX Viewer, it is important to keep the followings in mind:

- Calculating, rendering and storing 3D objects requires a significant amount of hardware resources.

- High-quality 3D reconstructions, which can be rotated and re-cut, require 40-100 slides.

- Naming the digital slides sequentially during scanning, will make it easier to keep them in order in MIRAX Viewer and in the 3D module.

- In order to create a 3D reconstruction that is proportionally accurate, do not remove slides from the 3D project even if they do not contain a specimen cut on the relevant part of the slide. When the module reconstructs the 3D object, equal slide thicknesses are used in the calculations; when a slide is missing from the original slide set, the module assumes the neighbors of the missing slide as adjacent slides and the proportions of 3D reconstruction will be distorted.

2 Installing the 3D Module for MIRAX Viewer

2.1 Requirements for Installing and Using the 3D Module for MIRAX Viewer

MIRAX 3D is not a standalone application: you need MIRAX Viewer to run it. Therefore, it is installed together with MIRAX Viewer, bundled into one executable.

2.2 Prerequisites

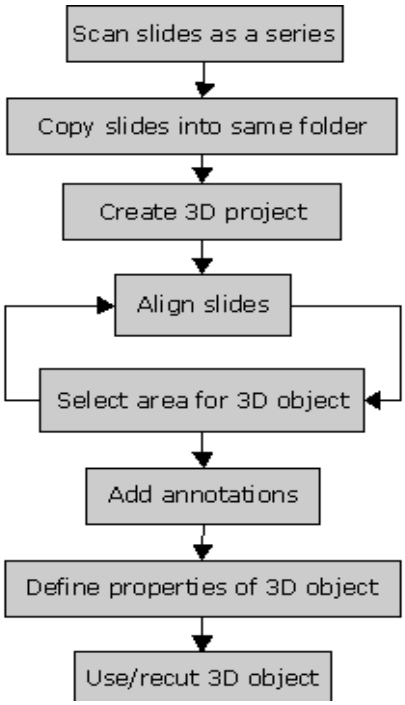
Prerequisites	**Description**
MIRAX Viewer version	1.11 Running on Microsoft Windows XP
Hardware	**Minimum**: Pentium P4, 3GHz, 1GB RAM **Recommended**: Pentium C2D (DualCore), 2 GHz, 2GB RAM
Disk space	MIRAX Viewer bundled with the 3D module **Minimum**: 110 MB

2.3 Installing the Application Bundle

- Click on the **setup.exe** file that you received on a DVD or downloaded in a compressed file format (*.zip).
- Follow the instructions of the installation wizard.

3 Creating a Virtual 3D Object

3.1 Overview



In the 3D module a wizard helps you in creating a virtual 3D image of the specimen from a series of 2D slides. The reconstruction process consists of the following 5 steps:

- **Disordered** - here you can delete unwanted slides from a series
- **Adjust** - aligns the tissue specimens on adjacent slides into a stack
- **2D View** - lets you review/save the complete aligned series
- **Exclusion** - lets you mask out the areas not to be reconstructed
- **3D View** - visualize, move, rotate, magnify and re-cut the 3D object

You can move forward and backward between the individual steps of reconstruction using the **<prev** and **next>** buttons at the bottom right corner. The step you are currently on is highlighted with red on the bottom status bar.

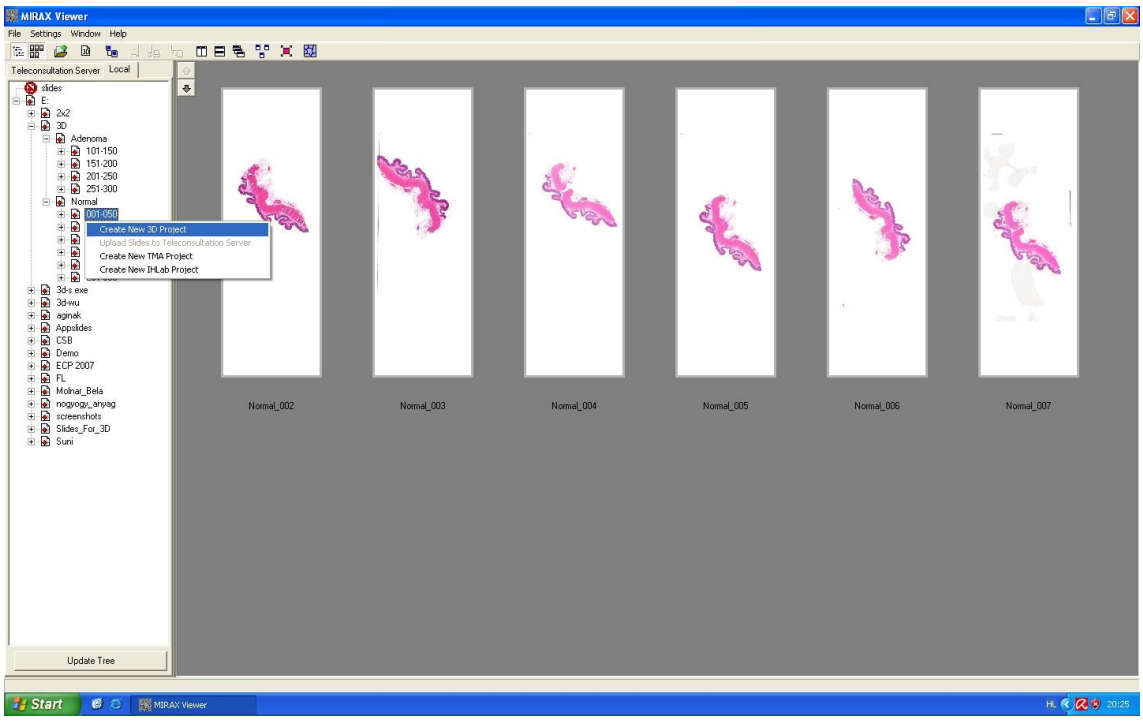
3.2 Using 3D Wizard

Place the digitalized series of slides into a folder from which they can be accessed by MIRAX Viewer. You will not have to preselect and delete the unwanted slides before launching the 3D application, you will be able to do all this within the 3D module in the Disordered step.

3.2.1 Starting a 3D Project

a. Launch MIRAX Viewer and start a new 3D project:

- From the menu, select File / New 3D.
- In the top toolbar, click on the button.
- For using the tree-view on the left panel, please, right-click the folder that contains the slides and then select **Create new 3D Project** (see below).



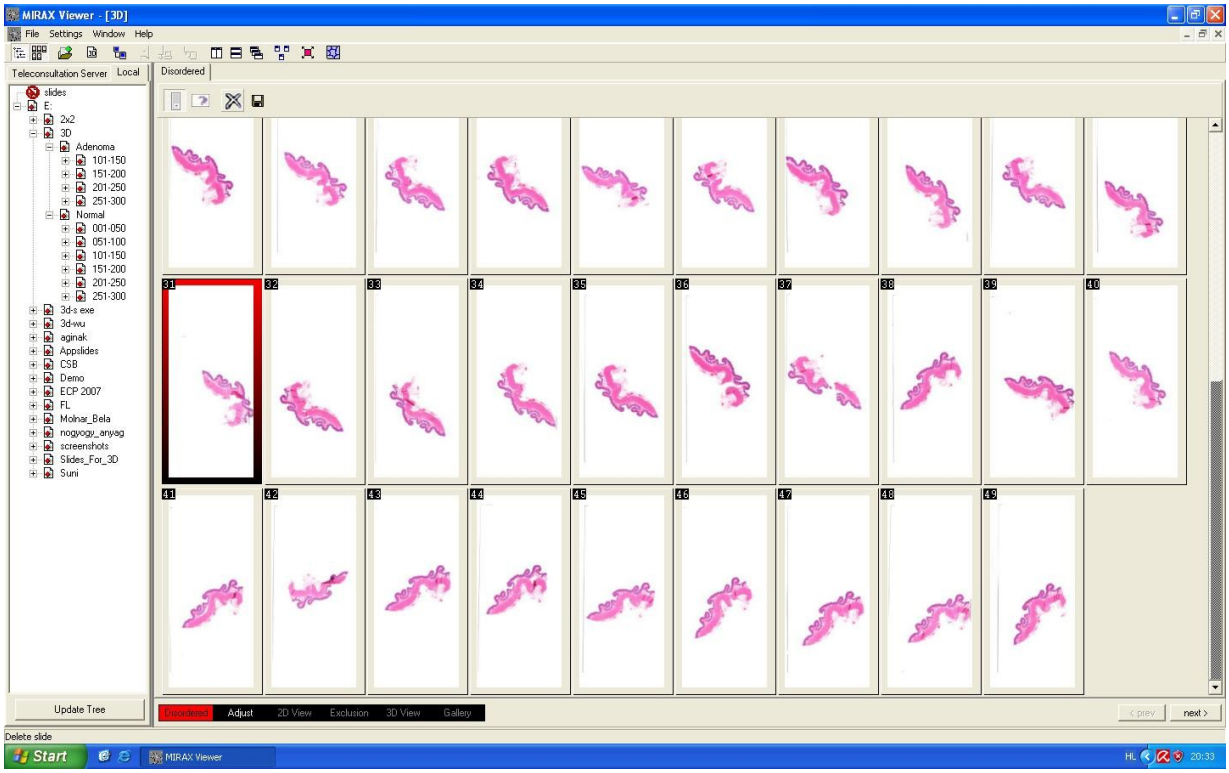
b. Launch a previously saved project by simply double-clicking on the name of the 3D project in MIRAX Viewer. All saved 3D projects are marked by icons in front of their names.

3.2.2 Disordered Tab

- With this step you can remove the slides that you do not want for the 3D image: highlight them and delete them one-by-one using the Delete key on the keyboard or with the button in the toolbar.

Warning!

If you drop a slide from the slide set, the 3D reconstruction will be distorted: when the module reconstructs the 3D object, equal slide thicknesses are used in the calculations; if a slide is missing from the original slide set, the module assumes the remaining neighbors of the slide as adjacent slides.



- Rearrange the slides as needed: click the slide that you want to move and drag it to its correct position in the series.

3.2.3 Adjust Tab

This step aligns the slides and forms a stack so that the corresponding points of adjacent tissue sections are matched and placed directly below each other. The alignment is usually a multi-step procedure. It can be performed **automatically** or **manually**. This step is necessary since the individual tissue sections within a series are not placed on the slides in the exact same orientation. So in order to match their relative position and orientation the subsequent slides need to be moved and/or rotated.

The selected slides for the reconstruction are listed in order on the right hand column. The two adjacent slides, the relative position of which is currently being aligned are pictured in the middle. (At the same time, there slides are highlighted with colored rims on the right column.)

a. **Automatic Alignment:** the software performs an automatic alignment based on the outline and the texture of the tissue sections. You can start this by simply clicking on the **Align** button on the top left.

   The algorithm by which the automatic alignment is performed can be selected by clicking on the **Settings** button right below the **Align** button. There are three options to choose from based on the shape of the tissue section. Examples are given to aid the selection of the most appropriate algorithm.

   Once the alignment is done, you can select the area of interest using the and the buttons. Select the button. Holding down the left mouse button, draw a box around your area of interest, than select it by clicking on the icon. This automatically zooms in the selected region and clears the remaining area of the slide.

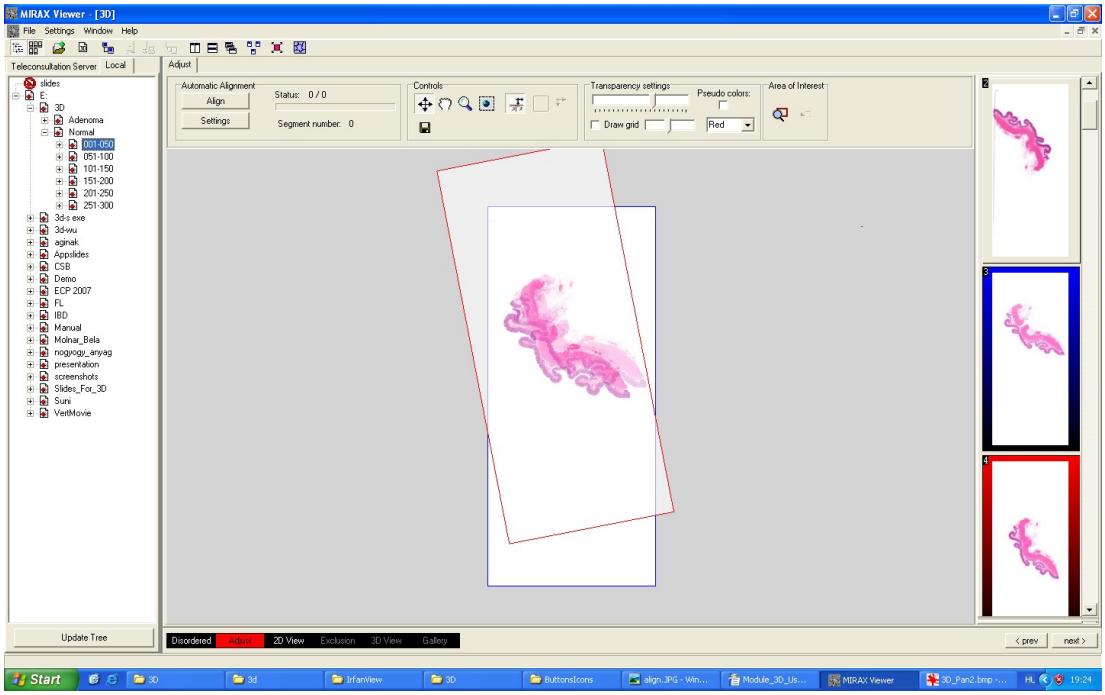
   Flip through the series and check to see the alignment of all slides with this higher magnification. If necessary click on the **Fine Align** button (in place of the Align button) to make the necessary corrections. This procedure can be repeated any number of times, until the sections in the stack match perfectly within the area of interest.

Tip!

Perform the alignment procedure sequentially and narrow in on your area of interest gradually with increasing magnification. Check the alignment of all slides every time. You can see a "shadow" from the previous slide if the slides are not sufficiently aligned.

b. **Manual Alignment:** you can also perform the alignment manually. Manual corrections may also be necessary following an automatic alignment when adjacent sections differ significantly in size, shape or texture.

The two adjacent slides, the relative position of which is being corrected, are highlighted in the right column and at the same time are shown in the middle with blue and red borders. Click on the icon. By holding down the left mouse button, you can move the top slide (red border) relative to the bottom slide (blue border) until they are perfectly aligned. If necessary you can also rotate the top slide relative to the bottom by holding down the right mouse button and moving the mouse.



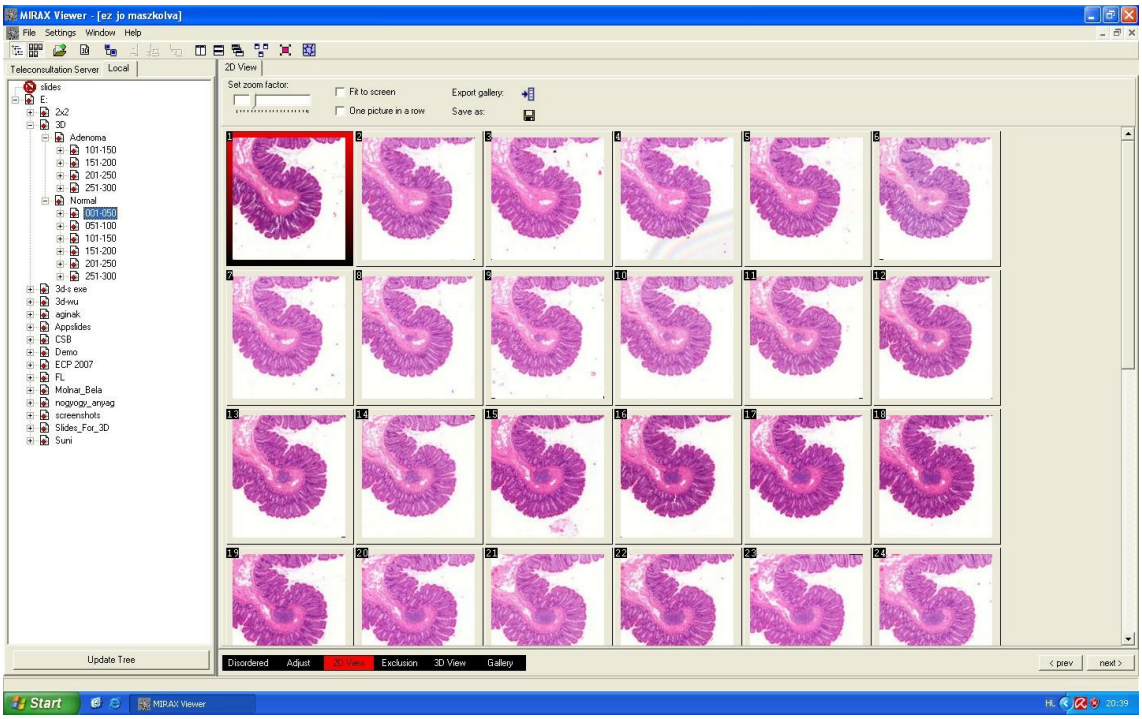
- Use the icon to zoom in on your area of interest.
- Use the icon to move both slides together (position them on the screen).
- You can save the project at any point by clicking on the icon.

  - In the **Transparency Settings** panel you can change the degree of the transparency, the assigned border colors and draw a scalable grid to aid manual alignment.

After completion of the alignment procedure, select your area of interest using the and the buttons, and click on N**ext>** to move to the following step.

3.2.4 2D View Tab

This step provides an opportunity to review the aligned series. The magnification can be adjusted using the sliding scale on the top left. To export the selected areas as an image library, please, click on , and to save the Project click on .

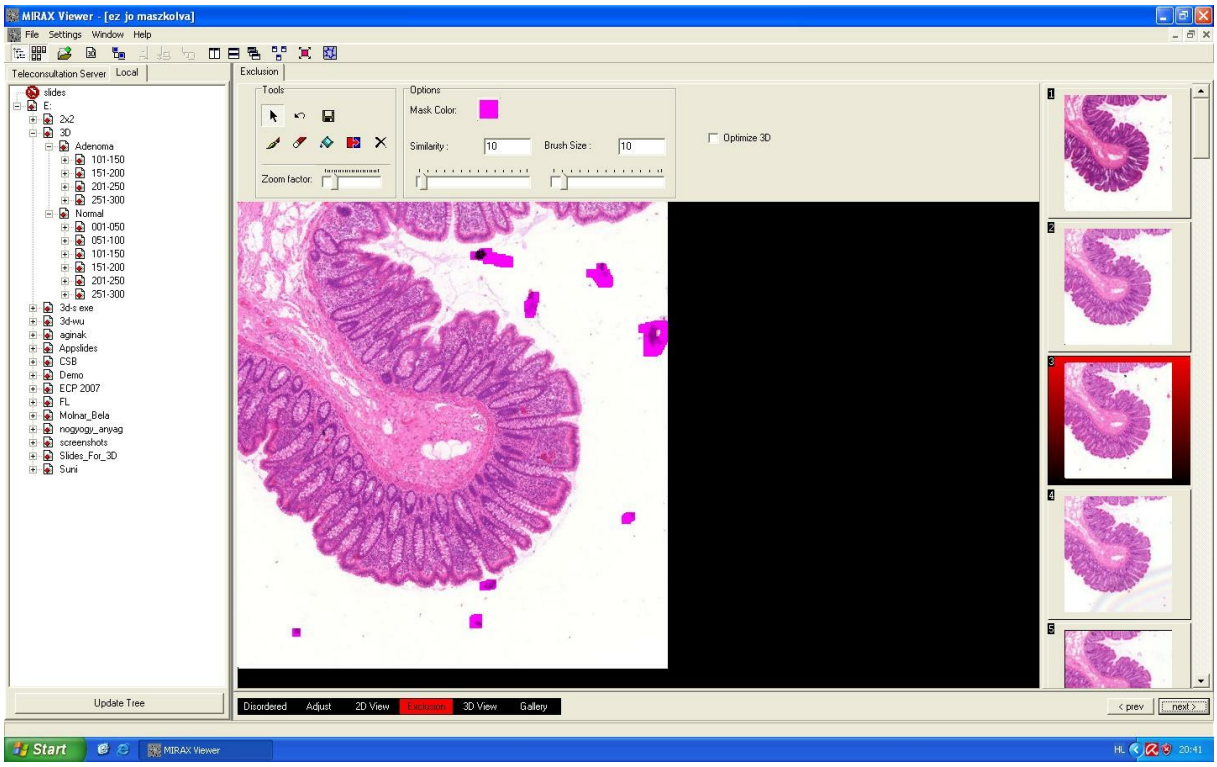


3.2.5 Exclusion Tab

This step allows for masking selected areas of each individual slide in the series which should not be present on the virtual 3D objects. This feature is most useful when the goal is to reconstruct smaller well circumscribed subsections within a tissue, such as a gland or vessel. As a result, only the unmasked regions from the slides will be rendered as the 3D object. Masking out the unnecessary tissue components greatly enhances the 3D output.

Tip!

This feature is a great help in cleaning off the artefacts that arise from histological tissue processing.



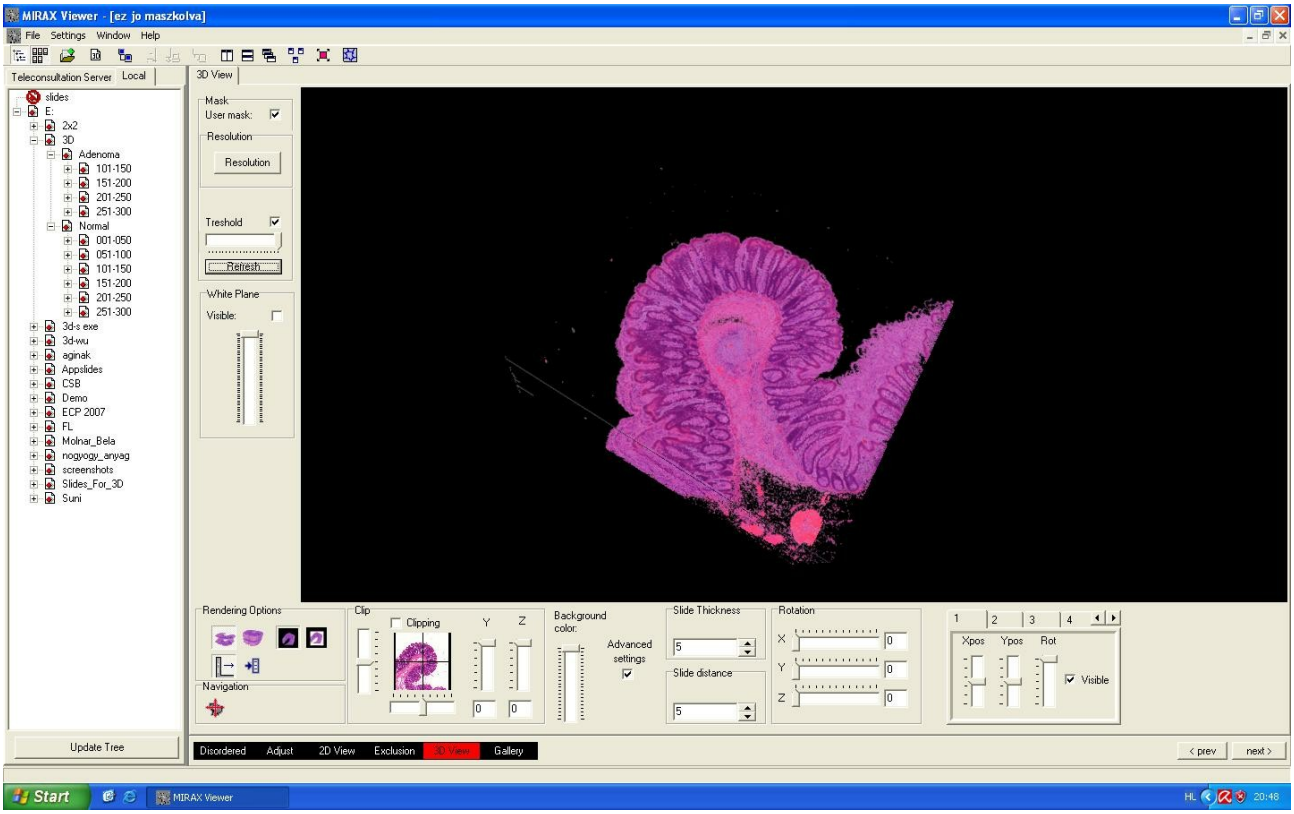
The drawing and filling tools for creating the mask can be found under the **Tools panel** on the left. The function of these tool are detailed in section 5.4.

3.2.6 3D View Tab

The virtual 3D object is created in this step. The object can then be freely rotated using the mouse and virtually re-cut. These virtual cuts allow for the investigation of the inner structure of the 3D object. By changing the **Threshold** in the left panel, the transparency of the object can be modified. This enhances the 3D appearance by eliminating the white cuboidal mask surrounding the object.

The bottom toolbar allows for the **clipping** (= **creating virtual cut surfaces**) of the object **in** all **three dimensions**. The advanced settings allow for adjusting the **slide thickness** and **slide distance** in order to recreate the dimensions of the original object more faithfully. There are also controls on this bottom toolbar for numerically adjusting the spacial position of the 3D object.

For information on the parameters, see section 5.5 3D View Tab on page 23.



3.2.7 Gallery Tab

Allows for generating an image series of virtual cut sections in any given plane from the 3D object. The number of slices to be created are to be set before this step.

4 Working with a 3D Object

➢ Start the project.

If you have not set up a project, follow the instructions in section 3.2 Using 3D Wizard (page 11).

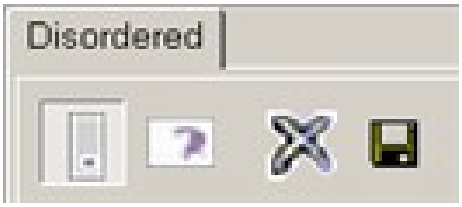
➢ Click the **3D View Tab**. Make sure that the **Advanced Settings** checkbox is selected.

The 3D object is displayed. You can manipulate it with setting the parameters numerically and/or with the mouse.

Rotating the 3D Object	• Use the 3 sliders for Rotation in Advanced settings to set the basic view of the object. • Enter the angle values manually next to the sliders. • Click into the image area, hold down the left mouse button and move the mouse to drag&rotate the object.
Moving the 3D Object	Click on the object, hold down the right mouse button and move the mouse.
Zooming the 3D Object	Hold down the center mouse button or the scroll wheel and move the mouse.

5 3D Module Reference

5.1 Disordered Tab



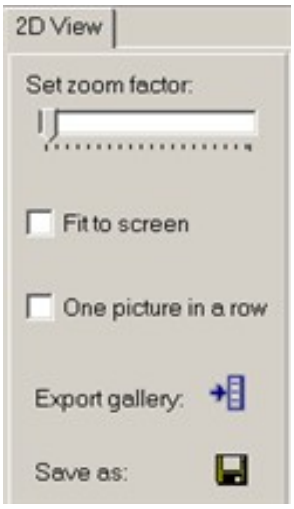
	Displays the entire virtual slide.
	Displays only the used part of the slide (where the tissue section is found).
	Deletes the slide from the current project. **The slide is not deleted from the folder.**
	Saves the 3D project under a new name.

5.2 Adjust Tab

Align / Fine Align	Automatically aligns the virtual slides: the slides are compared pairwise and the corresponding points of the cuts are matched. Before the slides are aligned, the button reads Align. The button changes to Fine Align for further alignment rounds.
	When this icon is pressed, you can move the active slide (the upper slide from the displayed slide pair).
	When this icon is pressed, you can move the work area, that is, both slides.
	When this icon is pressed, you can zoom in and out by holding down the left mouse button and moving the mouse up and down (zoom out and in, respectively)
	When this icon is pressed, you can select an area to narrow down the scope of the 3D reconstruction.
	When this icon is pressed, the selected area of all slide are aligned with each other consecutively. First the selection on slide 2 is aligned to the selection on slide 1, then the selection on slide 3 is aligned to the selection on slide 2, and so on. When this icon is not pressed, the and icon are active: you can select a primary area and separately the area for the 3D reconstruction.

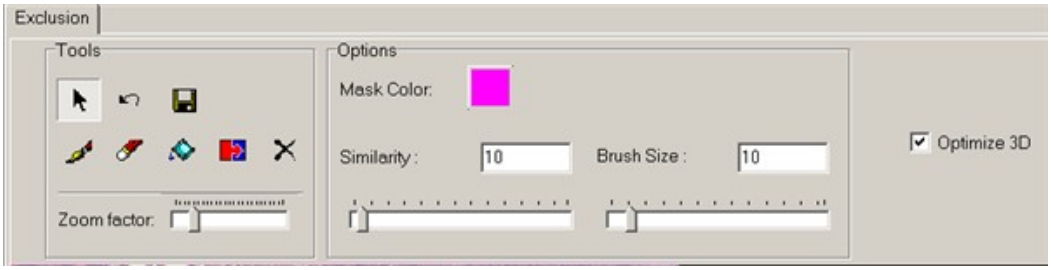
	When this icon is pressed, you can select a larger area.
	When this icon is pressed, you can select the area for the 3D reconstruction besides the primary area that you selected with .
Transparency settings	Gradually colors the slide in the front or the slide in the back so that you can check the accuracy of the alignment more easily.
Pseudo Colors	Displays the two slides with different colors.
Draw grid	Displays a grid over the workspace. You can change the grid step with a slider and select the grid color from a list of color names.
	Narrows the scope of the 3D reconstruction to the selected area. (area of interest)
	Undoes the last action.

5.3 2D View Tab



Set zoom factor	Defines the size of the images (the selected area on in slide).
Fit to screen **One picture in a row**	Define the size and the number of images displayed on your screen at the same time.
	Exports the images as an image gallery in an HTML file.
	Saves the project.

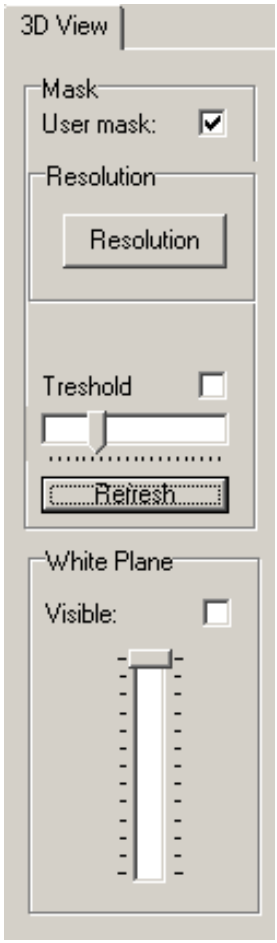
5.4 Exclusion Tab



	When this icon is pressed, you can move the current select on the screen.
	Undoes the last step.
	Saves the 3D project.
	Brush tool to mark an area.
	Eraser tool to remove the marking that you added with the brush or the filler tools.
	Filler tool to spread a color over the active area.
	Allows filling areas that have the same color in the digitized slide with the same color in the mask.
	Deletes the mask.
Zoom Factor	Zooms in and out the active image according to the slider setting. You can also jse the scroll button of your mouse for zooming.
Mask Color	Click the active color to change it from the color palette.
Similarity	Defines the color range that the filler tool uses.
Brush Size	Modifies the size of the brush tool.
Optimize 3D	Makes the reconstruction faster.

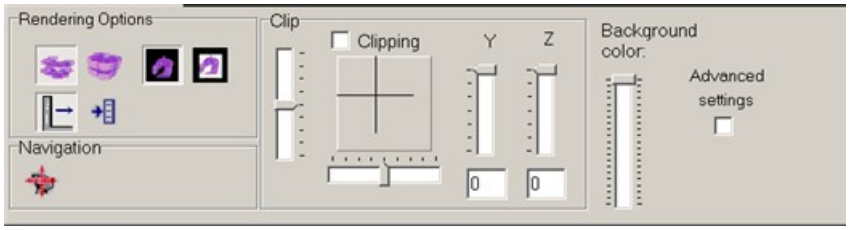
5.5 3D View Tab

5.5.1 Mask Definition Parameters



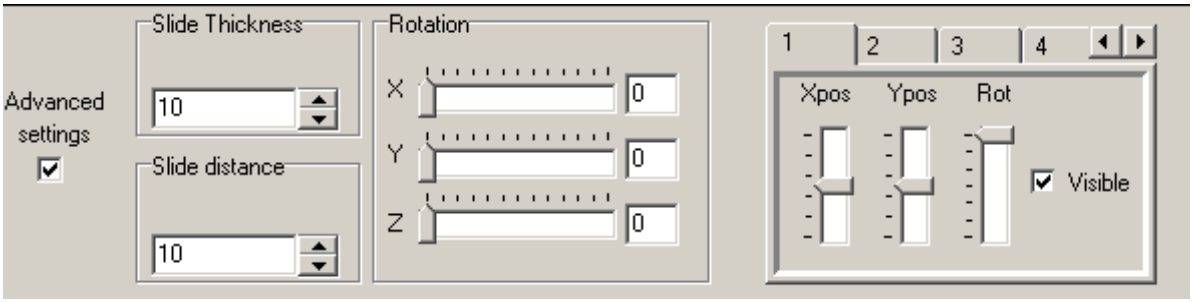
User masks	Shows or hides the mask
Resolution	Allows you to select one of the predefined resolutions for the reconstructed object.
Refresh	Refreshes the 3D object if the threshold settings were changed.
Visible	Cuts a white plane through the 3D object. You can change the Z-position for the sectioning plane.

5.5.2 Rendering Parameters



	Slide View Sections are displayed as slices.
	Object View Sections are displayed as blocks.
	Transparent View
	Normal View No transparency used when the sections are stacked and the 3D version of the specimen is reconstructed.
	Show or hides the panel of the mask definition parameter.
	Export the current view as an image embedded in an HTML file.
	Central view After rotation or zooming, returns the 3D object to its initial position.
Clip	Creates virtual sections from the 3D object. You can define the rotation (Z) and the angle (Y) of the sectional plane.
Backgr. Color	Sets the background color of the display window.

5.5.3 Advanced Settings for 3D Rendering



Slide Thickness	Sets the thickness of the virtual sections (in µm). If you want a contiguous 3D object, use the thickness of the specimen cuts as the value for Slide Thickness and set the Slide Distance parameter to 0.
Slide Distance	Sets the distance between the virtual sections (in µm).
Rotation	Rotate the virtual object with the sliders.
Visible	Allows settings per selection.

*